Figure 1

Annealing site 3

Sac I

Annealing site 4

Sal I

Neo^R

pDG2

Annealing site 2
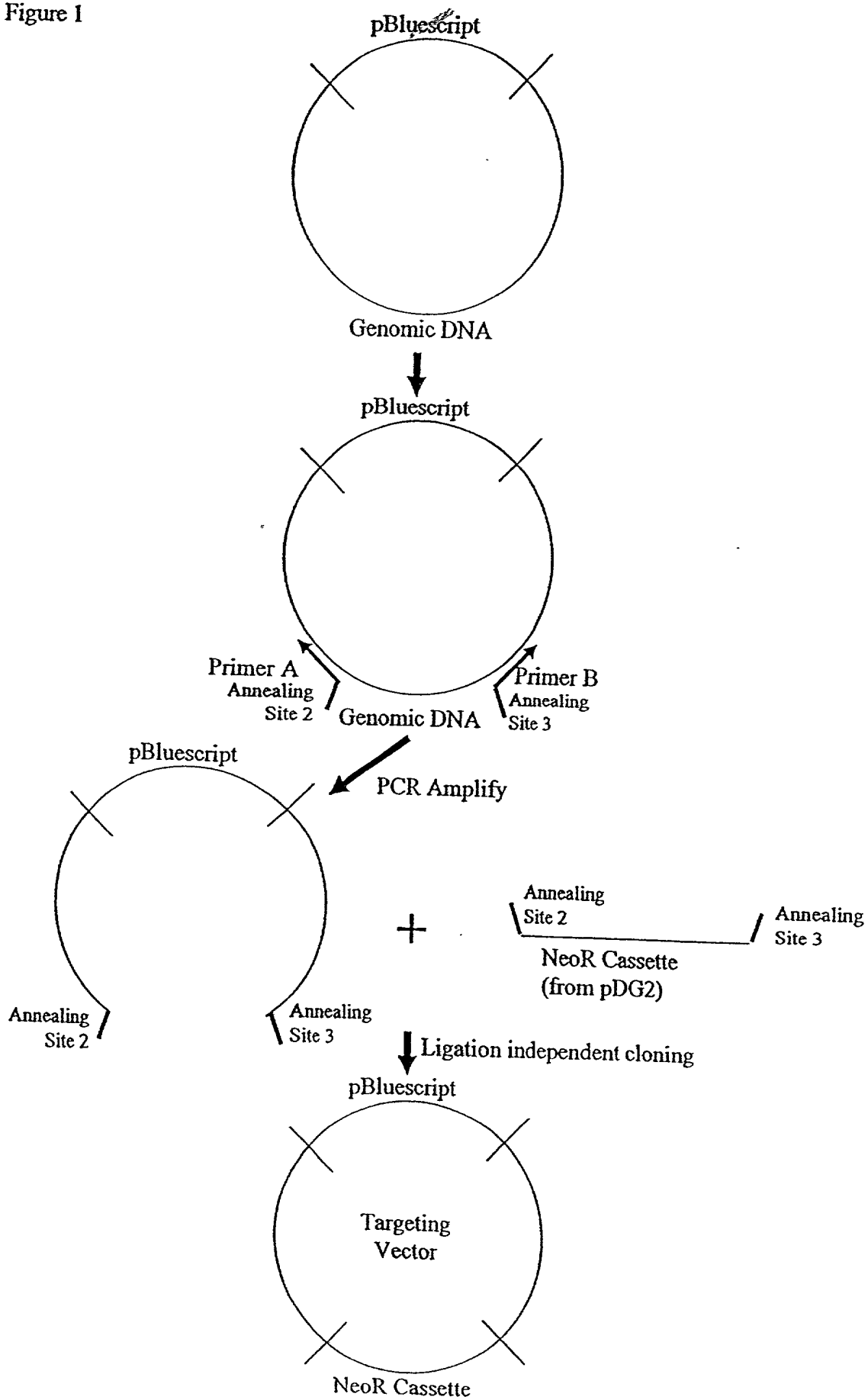
Sac II
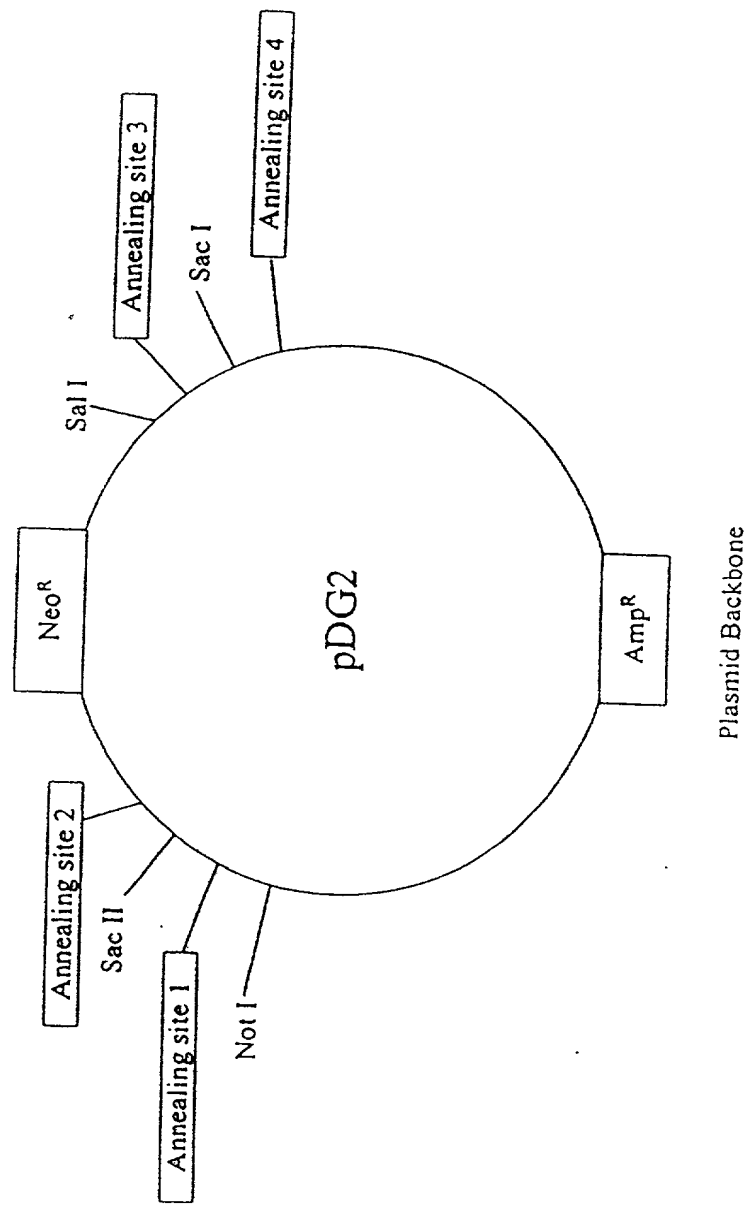
Annealing site 1

Not I

Amp^R

Plasmid Backbone

FIGURE 2A

pDG2:

```
GTTAACTACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATA
TGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTC
CGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGA
TGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCC
CCGAAGAACGTTCTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAA
GAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGA
TGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGA
TCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAG
CTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAAC
TGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGC
GCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCA
CTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAG
ACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTG
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TTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTAT
AAATCAAAAGAATAGCCCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTC
CAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCAAATCAAGTTTTTTGGGGT
CGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCGAACGTGGCGA
GAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACA
CCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAA
TCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTT
CTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAAC
TCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACC
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TCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCAC
ACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCG
AAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAAC
GCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCG
GAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTAATGTG
AGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATA
ACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTACGTAATACGACTCACTAGGCGGCCGCGTTTAAAC
AATGTGCTCCTCTTTGGCTTGCTTCCGCGGGCCAAGCCAGACAAGAACCAGTTGACGTCAAGCTTCCCGGGACGCGTGCT
AGCGGCGCGCCGAATTCCTGCAGGATTCGAGGGCCCCTGCAGGTCAATTCTACCGGGTAGGGGAGGCGCTTTTCCCAAGG
CAGTCTGGAGCATGCGCTTTAGCAGCCCCGCTGGCACTTGGCGCTACACAAGTGGCCTCTGGCCTCGCACACATTCCACA
TCCACCGGTAGCGCCAACCGGCTCCGTTCTTTGGTGGCCCCTTCGCGCCACCTTCTACTCCTCCCCTAGTCAGGAAGTTC
CCCCCCGCCCCGCAGCTCGCGTCGTGCAGGACGTGACAAATGGAAGTAGCACGTCTCACTAGTCTCGTGCAGATGGACAG
CACCGCTGAGCAATGGAAGCGGGTAGGCCTTTGGGGCAGCGGCCAATAGCAGCTTTGCTCCTTCGCTTTCTGGGCTCAGA
GGCTGGGAAGGGGTGGGTCCGGGGGCGGGCTCAGGGGCGGGCTCAGGGGCGGGGCGGGCGCGAAGGTCCTCCCCGAGGCCC
GGCATTCTCGCACGCTTCAAAAGCGCACGTCTGCCGCGCTGTTCTCCTCTTCCTCATCTCCGGGCCTTTCGACCTGCAGC
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ACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTC
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TGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGATG
ATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGT
GGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATG
GGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGT
TCTTCTGAGGGGATCGATCCGTCCTGTAAGTCTGCAGAAATTGATGATCTATTAAACAATAAAGATGTCCACTAAAATGG
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GTGGGGGTGGGGTGGGATTAGATAAATGCCTGCTCTTTACTGAAGGCTCTTTACTATTGCTTTATGATAATGTTTCATAG
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TAGCCTGAAGAACGAGATCAGCAGCCTCTGTTCCACATACACTTCATTCTCAGTATTGTTTTGCCAAGTTCTAATTCCAT
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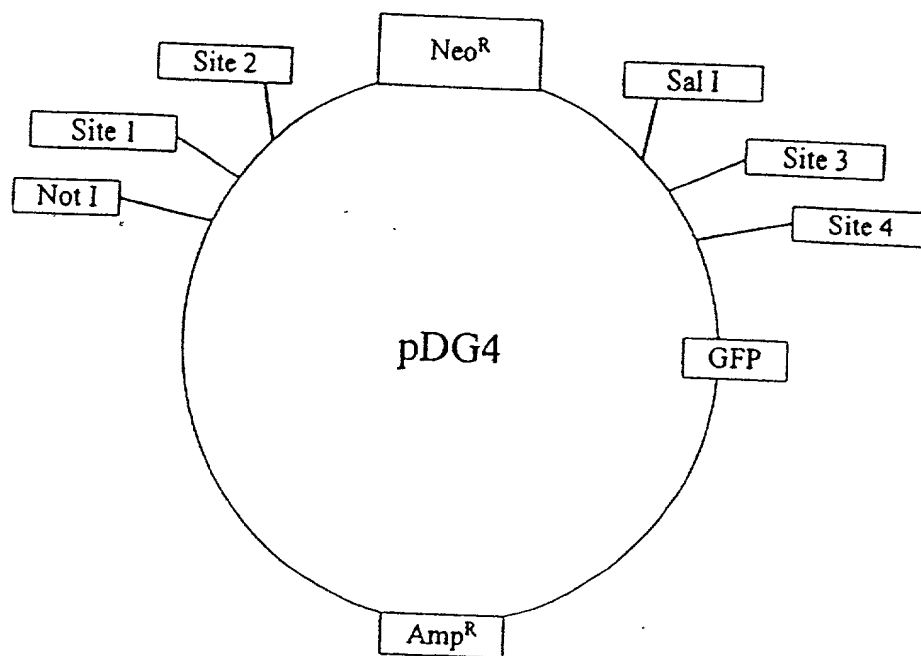
FIGURE 3A

pDG4:

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CTTTCCAATGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT
ACGCCCCCTATTGACGTCAATGACGGAAAATGGCCCGCCTGGCATTAAGCCCAGTACATGACCTTATGGGACTTTCCTAC
TTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGC
GGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGAC
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GGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATG
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GCGCTTTAGCAGCCCCGCTGGCACTTGGCGCTACACAAGTGGCCTCTGGCCTCGCACACATTCCACATCCACCGGTAGCG
CCAACCGGCTCCGTTCTTTGGTGGCCCCTTCGCGCCACCTTCTACTCCTCCCCTAGTCAGGAAGTTCCCCCCCGCCCCGC
AGCTCGCGTCGTGCAGGACGTGACAAATGGAAGTAGCACGTCTCACTAGTCTCGTGCAGATGGACAGCACCGCTGAGCAA
TGGAAGCGGGTAGGCCTTTGGGGCAGCGGCCAATAGCAGCTTTGCTCCTTCGCTTTCTGGGCTCAGAGGCTGGGAAGGGG
TGGGTCCGGGGGCGGGCTCAGGGGCGGGCTCAGGGGCGGGGCGGGCGCGAAGGTCCTCCCCGAGGCCCGGCATTCTCGCAC
GCTTCAAAAGCGCACGTCTGCCGCGCTGTTCTCCTCTTCCTCATCTCCGGGCCTTTCGACCTGCAGCCAATATGGGATCG
GCCATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACA
GACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGT
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CTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCT

TGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCG
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CCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTG
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CTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGGGGA
TCGATCCGTCCTGTAAGTCTGCAGAAATTGATGATCTATTAAACAATAAAGATGTCCACTAAAATGGAAGTTTTTCCTGT
CATACTTTGTTAAGAAGGGTGAGAACAGAGTACCTACATTTTGAATGGAAGGATTGGAGCTACGGGGGTGGGGGTGGGGT
GGGATTAGATAAATGCCTGCTCTTTACTGAAGGCTCTTTACTATTGCTTTATGATAATGTTTCATAGTTGGATATCATAA
TTTAAACAAGCAAAACCAAATTAAGGGCCAGCTCATTCCTCCCACTCATGATCTATAGATCTATAGATCTCTCGTGGGAT
CATTGTTTTTCTCTTGATTCCCACTTTGTGGTTCTAAGTACTGTGGTTTCCAAATGTGTCAGTTTCATAGCCTGAAGAAC
GAGATCAGCAGCCTCTGTTCCACATACACTTCATTCTCAGTATTGTTTTGCCAAGTTCTAATTCCATCAGAAGCTGACTC
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CCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCC
AGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTCGC
TTGGTAATAAAGCCCGCTTCGGCGGGCTTTTTTTT

FIGURE 3B (Continuted)

Annealing site	Sequence	Sequence after digestion
1	5' tgtgctcctcttggcttgcttccaa.... 3' 3' acacgagaggagaaaccgaacgaaggtt.... 5'	5' tgtgctcctcttggcttgcttccaa... 3' 3' tt... 5'
2	5' ctggttcttgtctggcttggcccaa.... 3' 3' gaccaagaacagaccgaaccgggtt.... 5'	5' ctggttcttgtctggcttggcccaa... 3' 3' tt... 5'
3	5' ggtcctcgctctgtgtccgttgaa.... 3' 3' ccaggagcgagacacaggcaactt.... 5'	5' ggtcctcgctctgtgtccgttgaa... 3' 3' tt... 5'
4	5' tttgcgtgtcctgtgtcgtcgaa.... 3' 3' aaacgcacaggacacagcagctt.... 5'	5' tttgcgtgtcctgtgtcgtcgaa... 3' 3' tt... 5'

FIGURE 4

| Annealing site	Sequence	Sequence after digestion
1	5' AAtgtgctcctcttggcttgcttCCGC 3' 3' Ttacacgaggagaaaccgaacgaagg 5'	5' AA 3' 3' Ttacacgaggagaaaccgaacgaagg 5'
2	5' AActggttcttgtctggcttggCCCGC 3' 3' Ttgaccaagaacagaccgaaccggg 5'	5' AA 3' 3' Ttgaccaagaacagaccgaaccggg 5'
3	5' AAggtcctcgctctgtgtccgttGAGCT 3' 3' Ttccaggagcgagacacaggcaac 5'	5' AA 3' 3' Ttccaggagcgagacacaggcaac 5'
4	5' AAtttgcgtgtcctgtcgtcgtcacagcagc 3' 3' Ttaaacgcacaggacacagcagc 5'	5' AA 3' 3' Ttaaacgcacaggacacagcagc 5'

FIGURE 5

FIGURE 6
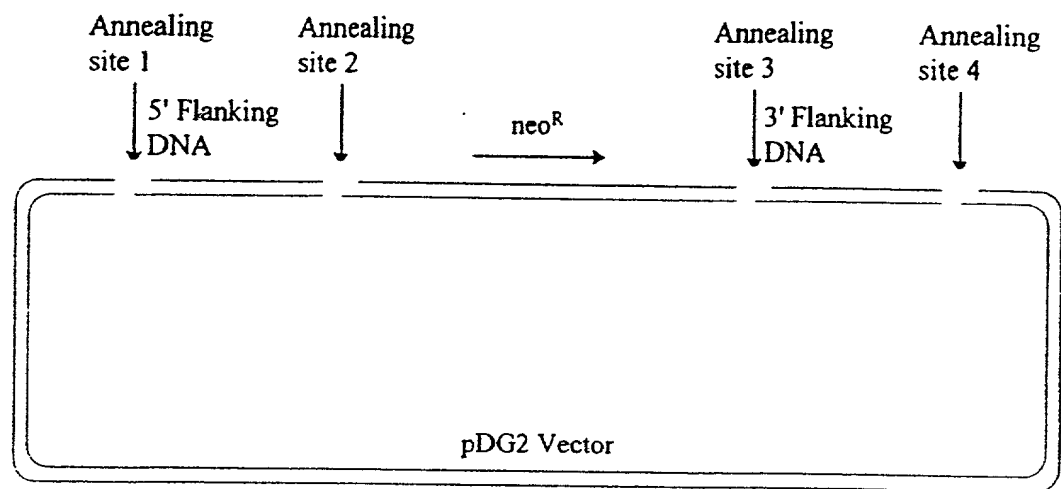
FIGURE 7
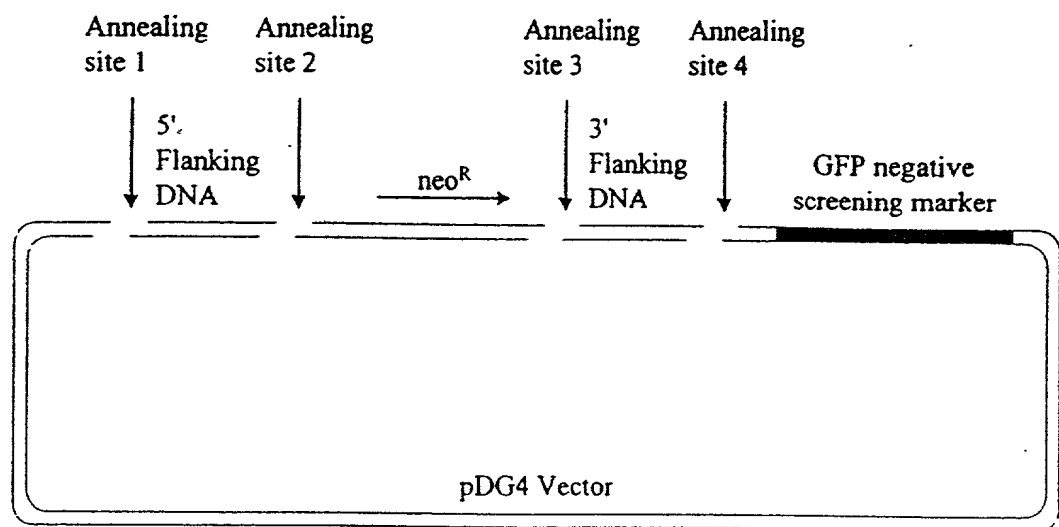
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AGAGGATCCAACAGGTGTGGGCCCCTCGCTCCAGTGCCGAGTGTGTGGGACAGCAGCAGTGGGAAACAT
TATGGCATCTATGCCTGCAATGGCTGCAGTGGCTTCTTCAAGAGGAGTGTGAGAAGGAGGCTCATCTACA
GGTGCCAAGTCGGGGCAGGGATGTGCCCAGTGGATAAGGCCCATCGCAATCAGTGCCAGGCCTGCCGGCT
GAAGAAGTGCTTACAAGCAGGCATGAACCAAGATGCTGTGCAGAATGAGCGCCAACCTCGGAGCATGGCT
CAGGTCCACCTGGATGCCATGGAAACAGGCAGTGACCCCCGATCAGAACCAGTGGTAGCCTCTCCTGCTC
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CAGCCTTATCACCGCCGAAACTTGTGCTAAACTGGAGCCAGAGGACGCTGAAGAGAATATTGATGTCACC
AGCAATGACCCCGAGTTCCCCGCATCCCCCTGCAGTCTGGATGGCATCCATGAGACATCTGCTCGCCTGC
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CTTGCTGGAAGAGGCATGGAATGAGCTTTTTCCTTCTTGGAGCCATACAGTGGTCTCTGCCCCTGGACAGC
TGCCCACTGCTGGCACCACCTGAGGCGTCCGGCAGCTCTCAGGGCAGGCTGGCCTTGGCCAGTGCAGAGA
CGCGCTTCCTGCAGGAAACCATCTCCCGGTTCCGAGCTCTGGCAGTGGATCCCACAGAGTTTGCCTGCCT
GAAGGCCCTGGTCCTCTTCAAACCTGAAACACGAGGCCTGAAGGATCCTGAGCACGTGGAGGCTTTGCAG
GACCAGTCCCAGGTGATGCTAAGCCAGCATAGCAAGGCTCACCACCCCAGCCAGCCTGTGAGGTTTGGGA
AATTGCTCCTCCTGCTCCCATCTTTGAGGTTCCTCACGGCTGAGCGCATTGAGCTTCTCTTCTTCAGAAA
GACCATAGGGAACACTCCGATGGAGAAGCTCCTGTGTGACATGTTCAAAAACTAGTTGGGAGTGCCAAGT
GTCCACAGGCACCCAGGGGGGCAGCACATCTTAGAAGCTAAATAGTTCCCTGCCTTTCTCAGCCAGTAAT
TCCACATTCAGGTATTCCTACCTAGCAGAAATTTCTCCCAAAATATATTATTGGCATATTCATTGCCATC
CTAATCTTAATACCCCTAACTCTGCTTGGGCAGTAGAATGCATGGATGCGTTGTTATATTCATAGGAGAA
ACAGCTTTGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
(SEQ ID NO:19  )
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Targeting Vector (5' arm; 200 bp flanking neo insert):

```
AGACTGAAAGACAGACAGACAGACAGACAGGGGGTTAAAGATGGATGCATCGGTTGGGCCCAGCAACT
TCTAGCAAGCAGGCTACCCTTAGGACCATCCATATCCGATGAGCTCTACAGTGGCTGCCTCCACTATG
CCTGTGTCTGTGGCGGCCTCCAAGAAGGAGTCTCCAGGTAGATGGGGCCTTGGAGAGGATCCAAC
(SEQ ID NO: 20 )
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Targeting Vector (3' arm; 200 bp flanking neo insert):

```
CTCCAGTGCCGAGTGTTTGGGGACAGCAGCAGTGGGAAACATTATGGCATCTATGCCTGCAATGGCTG
CAGTGGCTTCTTCAAGAGGAGTGTGAGAAGGAGGCTCATCTACAGGTGCCACAGCTCTGCCGGCCTG
CCCCGGTGTGCCTAGCACGGGTGGAGGGCGTTCAGGGAAAGCGGAAGACGAGACCAGGGCAAACA
(SEQ ID NO: 21 )
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FIG. 8